REMARKS

Claims 1, 2, and 7 have been amended to more particularly point out and distinctly claim that which Applicants believe to be the invention. Claims 8 and 9 have been canceled without prejudice. Reconsideration of the above identified application, in view of the above amendments and the following remarks, is respectfully requested. As of this amendment, claims 1-2, 4-7, 10-11 and 13 are pending in this application.

Claim 1 has been amended to recite a method for inducing T-cell tolerance to alloantigen-bearing cells *ex vivo* comprising purifying $CD4^+$ T-cells from donor tissue, irradiating alloantigen-bearing cells obtained from a recipient to deplete recipient T- cells, and producing a mixed lymphocyte reaction culture comprising the purified donor $CD4^+$ T-cells, irradiated, T-cell depleted alloantigen-bearing recipient cells, and an anti-gp39 antibody, then incubating the mixed lymphocyte reaction culture *ex vivo* for a sufficient time to render the donor $CD4^+$ T-cells substantially tolerant or non-responsive to said alloantigen-bearing cells, followed by assaying *ex vivo* for induction of donor $CD4^+$ T-cell tolerance or non-responsiveness.

Claim 2 has been amended to replace the "donor cells" as previously claimed in Claim 1 with "donor tissue" as now claimed in Claim 1. Support for amended Claims 1 and 2 can be found, e.g., on page 3, paragraphs 33, 36, and 38; and Examples 1 and 5 of the specification as filed (US 2002/0022020 A1).

Claim 7 has been amended to claim a 6 to 10 day incubation period for the mixed lymphocyte reaction culture of Claim 1. Support for this amendment can be found in Figures 1, 2A, and 2B, where measures of T-cell alloantigen hyporesponsiveness indicate that substantial T-cell tolerization occurs within this time period.

Rejection under 35 U.S.C. 103(a)

Claims 1, 2, 4-11 and 13 stand rejected as allegedly obvious over Noelle et al., U.S. Patent No. 5,876,718 ("Noelle") in combination with Riddell et al., 1990, J. Immunol Methods 128: 189-201 ("Riddell"), Rooney et al., U.S. Patent No. 5,962, 318 ("Rooney"), and Sykes et al., U.S. Patent No. 6, 006, 752 ("Sykes"). The Examiner alleges that Noelle combined with i) the irradiation of

antigen presenting cells in Rooney, ii) the culture of donor T-cells for treatment over varying lengths of time in Riddell, and iii) the monitoring of the induction of T-cell non-responsiveness *ex vivo* in Sykes renders the claims of the present invention obvious.

In order to further prosecution, while in no way agreeing with the Examiner's continued rejections, Applicants have amended the claims to clarify the subject matter of the claimed invention. Applicants respectfully submit that the claims as amended are not obvious in view of the combination of references cited in the office action, and the rejections as outlined in the Office Action are now moot.

The problem of Graft Versus Host Disease

Graft Versus Host Disease (GVHD) is a multi-organ system destructive process caused by the infusion of donor allogeneic T-cells into transplant recipients. The problem, at its core, is that donor T-cells must be prevented from triggering an immune response within the recipient against the recipient's own cells. Approaches to prevent this complication are needed. The two approaches used to date, the infusion of immune suppressive agents to decrease all immune responses in the recipient, or *ex vivo* attempts to remove all T-cells from the donor tissue, have significant negative side effects.

The invention of the instant application provides a novel treatment for GVHD

The present invention provides a successful method for treating donor T-cells *ex vivo*, to render such T-cells substantially non-responsive to recipient antigens. The disclosure of the invention describes a method for treating donor T-cells *ex vivo* with a gp39 (CD154) antagonist and recipient cells. The claimed method of the invention successfully renders donor T-cells substantially non-responsive to recipient antigens. The present invention thus provides an effective means of preventing or inhibiting GVHD responses that would otherwise potentially occur upon transplantation of donor tissues into a recipient.

The inventors have determined that the GVHD response can be controlled by tolerizing donor T-cells *ex vivo* in a specific manner. First, $CD4^+$ helper T-cells are removed and purified from the

donor; as an additional requirement, recipient cells are irradiated to remove recipient T-cells; then the purified T-cells are incubated in a mixed lymphocyte reaction culture with the irradiated recipient cells and a gp39 antagonist. Exposure to the recipient cells in combination with the gp39 antagonist causes any donor $CD4^+$ T-cells that recognize the recipient cells as foreign to become inactive. When treated in this manner, transplanted tissue does not cause a GVHD reaction in recipients.

Noelle, Rooney, Riddell and Sykes, alone or in combination, fail to teach or suggest all the limitations of the presently claimed invention

Noelle, Rooney, Riddell and Sykes fail to teach all the limitations of the invention as claimed. These references do not teach a mixed lymphocyte reaction culture comprising $CD4^+$ T-cells purified from donor tissue, combined with irradiated, T-cell depleted alloantigen-bearing recipient cells, and anti-gp39 antibody. The inventors have discovered that this unobvious combination successfully prevents GVHD-related health problems.

Noelle suggests *in vitro* tolerization of donor T-cells by incubating with recipient antigen-presenting cells (APCs; column 6, lines 50-55) or B cells (column 11, lines 22-27) and a gp39 antagonist. However, Noelle does not teach that the donor T-cells must be purified $CD4^+$ T-cells, or that the recipient APCs or B cells are first irradiated to deplete recipient T-cells. Thus, Noelle fails to teach crucial aspects of the invention. Therefore, in order for the combination of references to provide all limitations of the claimed invention, the use of purified $CD4^+$ T-cells from a donor, and the use of recipient APCs and/or B cells, which have been irradiated to remove recipient T-cells, must be taught or suggested by Rooney, Riddell, and/or Sykes. Applicants respectfully submit that this is not the case.

The use of purified $CD4^+$ T-cells

Culture of donor tissues containing donor T-cells is, according to the Examiner, taught by Riddell. The Examiner states that Riddell teaches "...the growth and expansion of antigen-specific T-cells in culture ... that can be employed for therapeutic use". Applicants agree with the

Examiner's assessment of Riddell. However, the growth and expansion of antigen-specific T-cells is the central problem in GVHD. As described below, the methods of Riddell are not applicable to the invention as claimed.

Applicants wish to distinguish the methods of Riddell from the instant invention. The method of culturing T-cells taught in Riddell involves collecting peripheral blood mononuclear cells and treating these cells in a mixed lymphocyte reaction (MLR) culture with antibodies to stimulate the growth of T-cells that are responsive to antigen. Application of anti-CD3 and anti-CD28 monoclonal antibodies to blood cells leads to expansion of T-cell populations. Riddell further teaches (see page 192, column 2, first paragraph) that culturing cells with anti-CD3 antibodies leads to cell populations which are enriched for alloreactive T-cells—the last thing one of skill in the art, seeking a treatment for GVHD, would want to encourage.

In contrast, the invention as claimed begins with the purification of $CD4^+$ T-cells from a donor. Purification of $CD4^+$ cells is not described in Riddell's method, and is not taught by Riddell or any combination of Riddell, Noelle, Rooney, or Sykes. Once purified, these cells are incubated with T-cell-depleted recipient B cells and anti-gp39 antibodies. The MLR of the invention, rather than stimulating T-cell growth, leads to T-cell anergy, because the method itself is different from the method of Riddell.

The Examiner contends that the "different endpoints" of the use of T-cells cultured using the methods of Riddell, relative to the treatment of donor T-cells in the current application, is irrelevant. However, the methods of Riddell lead to altered populations of T-cells in culture. Specifically, Riddell's method causes the activation and growth of immunoreactive T-cells. The teachings of Riddell would not and could not be applied to the invention as claimed because Riddell teaches a method to encourage the growth of immunoreactive T-cells, while the methods of the invention seek to ablate immunoreactive T-cell activity entirely. Therefore, the culture methods of Riddell would be detrimental to the invention as claimed in the instant application. The issue is not simply that the end-uses of the cultured T-cells are polar opposites. The issue is that the culture techniques themselves are opposite.

Contrary to the Examiner's assertion, Riddell is also inconsistent with any teachings in Noelle regarding the growth and expansion of T-cells in culture. Noelle does not suggest that T-cells should be cultured with anti-CD3 or anti-CD8 antibodies to encourage T-cell growth. Noelle suggests, in fact, that T-cells cultured *ex vivo* with T-cell-directed antibodies will cause T-cell depletion, not T-cell growth (Noelle, column 10, second paragraph). This point is discussed further *infra*. One of ordinary skill in the art would not have a motivation to combine Riddell and Noelle to arrive at the present invention. Any attempt to apply the teachings of Riddell to Noelle would theoretically result in either an exacerbation of transplant rejection by generation of an army of transplant-specific T-cells (following Riddell), or alternately, no T-cells at all (following Noelle). A reference which teaches an opposite concept teaches away, and cannot be properly combined to make an obviousness rejection. See *In re Lundsford*, 148 U.S.P.Q. 721, 726 (CCPA 1966).

There is no suggestion in Noelle, Riddell, Rooney, or Sykes to use purified donor $CD4^+$ T-cells in an MLR to tolerize donor T-cells to recipient antigens and prevent GVHD. Therefore, the use of purified donor $CD4^+$ T-cells in the manner of the invention as claimed is not obvious in view of these references.

Applicants wish to reiterate that an important factor in the success of the invention as claimed is not whether T-cells are cultured, nor that an MLR culture is created. The success of the invention rests in large part on the unobvious MLR combination of purified donor $CD4^+$ T-cells, with irradiated, T-cell-depleted recipient cells, and anti-gp39 antibody, that is useful for the treatment of GVHD. One could imagine that an MLR containing any type of cell may have been described in the art; however, absent a suggestion to produce this specific combination, a simple description of an MLR not containing any particular cell type, and without providing the exact elements of the invention as claimed and a motivation to combine the references describing the elements, cannot render the invention obvious.

Irradiating tissues obtained from a recipient to remove recipient T-cells, while leaving other recipient lymphocytes intact

The Examiner states that Rooney provides basic principles and practices of cell culture, including irradiation of antigen presenting cells (APCs). Applicants again reiterate the point presented *supra* that the prior existence of cell culture techniques, and methods for expanding T-cells in culture, does not impact the patentability of the invention as claimed.

Rooney does not teach irradiation to deplete T-cells. Rather, Rooney teaches irradiation to deplete APCs, and to assist T-cell growth in culture. Applicants respectfully remind the Examiner of the distinction between APCs and T-cells, as outlined in Rooney. An antigen presenting cell is defined as "an immune accessory cell that participates in antigen-inductive events, and includes mononuclear phagocytes, dendritic cells, and B cells" (Rooney, column 10, line 66 to column 11, line 2). Effector cells are "cells of the immune system that mount responses to protect individuals from pathogens" and encompass T-cells (Rooney, column 11, lines 36-42), which are cells of a lineage distinct from APCs.

Rooney teaches irradiation of cells to prevent proliferation of APCs and encourage the growth of T-cells. Rooney does not teach irradiation to deplete T-cells, unlike the method of the invention as claimed. Rooney teaches that "[i]rradiation of APCs prevents their [APC] proliferation, thus ensuring that only antigen-specific effector cells [e.g., T-cells] are selected in the culture." *See* Rooney at columns 14-15, overlapping paragraph. Thus, Rooney teaches irradiation of APCs to deplete APCs and encourage the growth of immunoreactive T-cells. From the teachings of Rooney, as irradiation assists in selection for T-cells, irradiation would not be useful for the removal of T-cells from a population of cells. In contrast, the invention as claimed utilizes irradiation to eliminate immunoreactive recipient T-cells, while keeping APCs and other cell types intact. Therefore, Rooney teaches a method which is not in accord with the methods of the invention as claimed. One of skill in the art seeking to eliminate T-cells would not use the methods of Rooney to achieve that end.

The focus of Rooney is similar to Riddell: to *stimulate* an immune response to specific antigens for adoptive transfer, which is useful to treat infections in immunocompromised

individuals, or to treat tumors. But this is not simply a matter of different endpoints for these references, as the Examiner suggests; different endpoints are achieved by different methods. These references fail to make obvious the methods of the invention because they do not teach the methods of the invention.

The Examiner states that Noelle teaches depletion of T-cells. The Examiner also indicates that Rooney is consistent with the teachings in Noelle for growth of T-cells in culture and manipulation of APCs. Applicants disagree with this assessment, particularly for depletion of T-cells. The irradiation of APCs in Rooney bears no resemblance to the method of T-cell depletion as suggested in Noelle. Rooney teaches a method to encourage the growth of T-cells and prevent proliferation of APCs by irradiating cell cultures. Noelle does not teach irradiation to remove T-cells; rather, Noelle teaches that T-cells may be removed from a mixed population of cells by treating the cells with an anti-T-cell antibody (specifically, anti-Thy1.1 and anti-Thy1.2) and complement (Noelle, column 10, lines 34-37). Noelle does not suggest that T-cells can be depleted by any means other than treating with anti-T-cell antibody. Further, Sykes is in agreement with Noelle that anti-T-cell antibodies lead to T-cell depletion (Sykes, column 10, lines 25-27). Rooney does not suggest any method for T-cell-depletion at all.

Having read the teachings of Rooney, Noelle, and Sykes, and with the understanding that irradiation is an appropriate method to prevent proliferation of APCs (Rooney), while treatment with anti-T-cell antibodies and complement is an appropriate way to remove T-cells while leaving APCs intact (Noelle and Sykes), the skilled artisan seeking to eliminate T-cells would conclude that irradiation of cells would result in a cell population depleted of APCs but enriched for immunoreactive T-cells, and so would not irradiate recipient tissue, but rather would apply anti-T-cell antibodies to recipient tissue. Not only does Noelle combined with Rooney and Sykes fail to teach irradiation to remove T-cells, but the combination in fact teaches away from this method. Riddell is wholly silent on this issue. Therefore, the combination of Noelle, Riddell, Rooney, and Sykes cannot make obvious the use of irradiation of recipient cells to deplete recipient T-cells.

Adding irradiated recipient cells and gp39 antibody to the donor CD4$^+$ T-cell culture to create a mixed lymphocyte reaction

As discussed *supra*, Noelle combined with Riddell, Rooney, and Sykes fails to teach an MLR comprising CD4$^+$ T-cells purified from donor tissue, combined with irradiated, T-cell-depleted alloantigen-bearing recipient cells, and anti-gp39 antibody. The novelty of the invention does not lie in culturing T-cells, or in creating an MLR. The invention teaches an MLR comprising elements that have not previously been combined in an MLR, with an end result that successfully treats GVHD. There is no motivation in the references cited to create this particular MLR comprising purified CD4$^+$ cells, or to add to the MLR irradiated T-cell depleted recipient cells, nor is there teaching to combine these particularly treated cells with anti-gp39 antibody. Therefore, the invention as claimed is not obvious in light of these references.

Incubating components for sufficient time to tolerize T-cells and assaying ex vivo for induction of T-cell tolerance

The Examiner contends that Noelle renders obvious the time ranges for culturing the T-cells as per instant claims 6 and 7. The Examiner cites the Examples in Noelle as evidence of knowledge in the art for culturing T-cells. Applicants respectfully direct the Examiner's attention to the fact that the Examples in Noelle do not give time frames for cell culture, but rather for treatment periods in *in vivo* rat studies. Thus, Noelle does not make obvious any time frame for incubating the MLR of the invention as claimed. Further, although the concept of incubating an MLR is not novel, the claimed time range of 6-10 days for the novel MLR of the invention is not disclosed in the combination of references cited, as the references cited do not teach the MLR of the invention.

The Present Invention Describes an Unexpected Result in an Unpredictable Art

The Examiner contends that Noelle teaches T-cell non-responsiveness to desired alloantigens with anti-gp39 antibodies and antigen presenting cells *in vitro*, before transfer of a transplant to a recipient *in vivo*. Applicants submit that Noelle, at best, suggests part of a potential method for inducing T-cell tolerance *in vitro* using anti-gp39 antibody. However, either alone or when combined with the cited references, Noelle fails to teach or suggest the complete method as claimed to successfully obtain T-cell tolerance or non-responsiveness of donor T-cells to desired alloantigen-bearing cells *ex vivo*.

Applicants respectfully submit that the invention of the amended claims is not rendered obvious by Noelle in combination with any or all of Riddell, Rooney and Sykes. Accordingly, Applicants respectfully request withdrawal of these rejections.

CONCLUSION

In view of the above amendments and remarks, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

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